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Metabolic profiling of four South African herbal teas using high resolution liquid chromatography-mass spectrometry and nuclear magnetic resonance

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Caffeine (PubChem CID: 2519)
Mangiferin (PubChem CID: 5281647)
Chrysosplenetin (PubChem CID: 5281608)
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ABSTRACT

Increased preference to herbal drinks has led to global interest in the use and production of different plant species for the preparation of various drink formulations. Medicinal properties derived from bioactive compounds remain the main driver of choice for herbal teas. This study determined the chemical variation in honeybush, rooibos, special and bush tea, profiled compounds responsible for such differences and compared their peak areas. Nuclear magnetic resonance and high resolution liquid chromatography-mass spectrometry were used to determine compound variation and profiling. Principal component analysis and partial-least square multivariate statistical analysis showed distinct differences ($P < 0.05$) between the different types of herbal teas. Detected compounds included flavonoids, phenolics, lignans, megastigmane glycoside, most of which possess health benefits. The findings showed that South African herbal teas could play a vital role as health promoting drinks, and that bush tea and special tea are phytochemically comparable with other commercialized herbal teas.

1. Introduction

Tea is the most widely consumed beverage in the world, second only to water. There are two distinct forms of tea, namely tea made from the leaves of the plant *Camellia sinensis* and herbal teas sourced from different plant species (Mandiwana, Panichev, & Panicheva, 2011). Herbal teas are made from one or more herbal substances intended for oral aqueous consumption. These substances include leaves, flowers, seeds, barks, roots and fruits of medicinal plants (Pohl et al., 2016). The South African herbal tea market is dominated by rooibos (*Aspalathus linearis* Burm.f. R.Dahlgren and honeybush *(C. intermedia* E.Mey and *C. subternata* Vogel*) C. subternata* Vogel*)* (Sissing et al., 2011). Rooibos tea was first discovered by the Khoi people indigenous to the Cape region of South Africa, though it now has a country-wide and indeed a global distribution, largely as a result of its use for the treatment of allergy, stomach cramps, eczema and nappy rash (Joubert & Schultz, 2012). Honeybush tea is also very popular with consumers and has traditionally been used as a medicinal concoction for soothing coughs, alleviating bronchial complaints and alleviating menopausal symptoms in women (Kamara, Brand, Brandt, & Joubert, 2004). The major compounds found in these teas are mangiferin, isomangiferin, eriocitrin, hesperidin, potassium, calcium, magnesium, phosphorus, isokuranetin,

Abbreviations: ^{1}H NMR, proton nuclear magnetic resonance; UPLC-Q-TOF/MS, ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry; COX-2, cyclooxygenase-2; mRNA, messenger ribonucleic acid; $PPAR_\gamma$, peroxisome proliferator-activated receptor gamma gene; NFκB, nuclear factor κ-light-chain-enhancer of activated B cells; D_2O, deuterated water; TSP, trimethylsilylpropionic acid; KH_2PO_4, potassium dihydrogen phosphate; OPLS-DA, orthogonal projection on latent structure-discriminant analysis; CSIR, council for scientific and industrial research; PCA, principal component analysis; PLS-DA, partial least-squares discriminant analysis; RT, retention time; KEGG, Kyoto encyclopedia of genes and genomes; ChEBI, chemical entities of biological interest

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dihydrochalcones, aspalathin, nothofagin, orientin, vitexin, and rutin (du Preez, de Beer, & Joubert, 2016; Olivier, Symington, Jonker, Rampedi, & Van Eeden, 2012; Schloms & Swart, 2014).

There are, however, other teas like bush tea (*Athrixia phylicoides* DC.) and special tea (*Monsonia burkeana* Planch. ex Harv.) which are popular among indigenous South Africans although they are not yet commercialized (Tshivhandekano, Ntushelo, Ngezimana, Tshikalange, & Mudau, 2014). Bush tea is a plant species of the genus *Athrixia* and is used to cure boils, acne, skin eruptions and coughs (Mudau, Mogotlane, Mashela, & Soundy, 2008). This tea contains, amongst other active ingredients, 3-0-demethyldigicitrin, 5,6,7,8,3′,4′-hexamethoxyflavone, quercetin, protocatechuic, p-coumaric, quercetin- 3′-O-glucoside, kaempferol, apigenin and chlorogenic acids (Mcgaw et al., 2013; Reichelt et al., 2012). The absence of caffeine or pyrrolizidine alkaloids makes bush tea a healthy alternative drink (McGaw, Steenkamp, & Eloff, 2007). Special tea is found in the southern African informal markets. The herb and roots of special tea are used as a remedy for colds and inflammation, diarrhoea and dysentery (Van Wyk, 2008). Furthermore, special tea is used to cure various sexually transmitted diseases, for blood cleansing, and to treat erectile dysfunction (Mamphiswana, Mashela, & Mdee, 2011). Some studies, however, have reported high phenolic content and the presence of chloride, copper, iron, zinc, magnesium and boron (Mamphiswana, et al., 2011; Tshivhandekano et al., 2014).

The beneficial effects of teas lie in the health promoting properties of their bioactive compounds. For instance, mangiferin, a compound found in honeybush tea, has been reported to hinder the expression of COX-2 by increasing mRNA expression of the Peroxisome Proliferator-Activated Receptor gamma ($PPAR_{\gamma}$) gene and down regulating the Nuclear Factor κ-light-chain-enhancer of activated B cells (NFκB) (Gold-Smith, Fernandez, & Bishop, 2016). Further studies on identifying plant metabolites could prove to be useful in disease prevention and treatment. Although data on the phytochemicals present in honeybush tea, bush tea and rooibos tea are available, there is a paucity of information on compounds available in special tea and, to date, no study has explored and compared all four herbal teas using ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF/MS). The aim of this study, therefore, was to evaluate the metabolite profiles of bush tea, special tea, honeybush tea and rooibos tea using proton nuclear magnetic resonance (^{1}H NMR) spectroscopic analysis and a non-targeted reversed phase UPLC-Q-TOF/MS approach, and to profile compounds responsible for their chemical profile similarities and dissimilarities.

2. Materials and methods

2.1. Sample collection and preparation

Two South African non-commercial herbal teas, bush tea (*Athrixia phylicoides*) and special tea (*Monsonia burkeana*) were collected from the experimental farm owned by Agricultural Research Council in Roodeplaat, Gauteng province and Hartbeespoort in Northwest province, respectively. All voucher specimens were deposited in the South African National Biodiversity Institute, National Herbarium, special tea (GENSPEC number 3925000) and bush tea (GENSPEC number 9055000).

Rooibos (*Aspalathus linearis)* and honeybush (*Cyclopia* species) tea were bought from a local supermarket in Johannesburg, South Africa. The leaves and twigs of special tea and bush tea were oven-dried at 50 °C for 48 h (Mamphiswana et al., 2011). Samples were then ground to powder using a Sample Mill Grinder (Tecator Knifetec 1095, Foss Tecator, Sweden) and stored in glass jars in the dark at room temperature until further analysis.

2.2. Chemicals and reagents

All standard reagents and chemicals, D_2O (99.9%), and TSP (97%) and methanol D_2, formic acid, were purchased from Sigma-Aldrich (St. Louis, USA). Liquid chromatography–mass spectrometry grade methanol and acetonitrile (99.9%) were purchased from Sigma-Aldrich (St. Louis, USA). Ultra pure water was obtained from the Milli-Q reagent water purification system (Millipore, Bradford, MA) at UNISA.

2.3. Analysis of the teas' metabolite profiles

2.3.1. ^{1}H NMR analysis of the selected teas

Fifty mg (50 mg) of oven-dried plant materials of the four South African herbal teas were each placed in separate 2 mL Eppendorf tubes followed by the addition of 0.75 mL Methanol D4 (CH_3OH-d_4) and 0.75 mL KH_2PO_4 buffer, prepared by mixing trimethylsilylpropionic acid sodium salt (TSP) with deuterated water (D_2O) containing 0.1% (w/w) TSP at pH 6.0. The mixtures were vortexed for one minute, sonicated for 20 min and then centrifuged at 11 337*g* for 20 min. The supernatants (approximately 750 µL) were transferred into 5 mm NMR tubes. NMR spectral data were obtained using a Varian 600 MHz spectrometer (CSIR, Pretoria) at a proton NMR frequency of 599.74 MHz. to suppress the water signal, A PRESAT pulse sequence was applied. Sixty-four (64) scans were acquired with 60-degree pulses at an acquisition time of 1.817 s. The spectral width was 18028 Hz with a relaxation delay of 4 s, and line broadening of 0.2 Hz before Fourier transformation.

All NMR spectra were phase and baseline corrected using MestReNova 10.01 (Mestrelab Research). The spectral regions (0–10.00 ppm) were divided into 0.04 ppm bins, converted to ASCII format and imported to Microsoft® Excel 2010. The resulting Microsoft Excel file was imported to SIMCA version 14.0 (Umetrics, Umeå, Sweden). Principal component analysis (PCA) score plots and partial least-squares discriminant analysis (PLS-DA) score plots were performed using Simca-P 14.0 software (Umetrics AB, Umeå, Sweden) after removal of outliers. To provide comparative interpretations and visualization of the metabolic differences among the individual teas, principal component analysis (PCA) and orthogonal projection on latent structure-discriminant analysis (OPLS-DA) were applied to the NMR spectrum data set (Fig. 1). Variation of data was explained by the greater variance (PC1) and the least variance (PC2). In cases where clear separation was not attained, OPLS-DA model was used to incorporate an orthogonal signal correction filter into the PLS model. However, the use of OPLS-DA model requires validation as it tends to over-fit models to the data (Worley & Powers, 2013).

The quality of the models was described by R^2X and Q^2 values. R^2X shows the proportion of variance in the data explained by the models and indicates goodness of fit. The value closer to 1 indicates the goodness of fit. Q^2, on the other hand, shows the proportion of variance in the data predictable by the model and indicates predictability (Hu et al., 2015).

2.3.2. LC-QTOF/MS analysis of selected teas

The water extraction method was used for preparation of all samples. In brief, 4 g of each tea sample was placed in a 50 mL Eppendorf tube followed by addition of 40 mL of boiled (100 °C) deionised water to each sample, as described by Eloff (1998). The mixtures were vigorously shaken, sonicated and then centrifuged; each step lasting 10 min. The resultant liquid was filtered into a beaker through Whatman No. 1 filter paper (11 µm pore size). The extraction was repeated using 20 mL and 10 mL of boiled deionised water on the same plant material. The filtered tea extracts were poured into Eppendorf tubes, stored at −80 °C overnight, and then freeze-dried. Ten mg portions of each freeze-dried sample (five replicates for each of the four teas) were placed into 2 mL Eppendorf tubes and dissolved by adding 1 mL formic acid (0.1%) in methanol. The samples were then vortexed